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Lymphoma With Multi Gene Rearrangement on the Level of Immunoglobulin Heavy Chain, Light Chains, and T-Cell Receptor β Chain

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A unique case with diffuse mixed malignant lymphoma was investigated for gene rearrangement on the level of T-cell receptor (TCR), heavy chain immunoglobulin (Ig), and both light chains. Cell phenotype was examined with immunofluorescence techniques using antibodies against surface immunoglobulins (Slg) and the kappa and lambda light chains. Monoclonal antibodies were used against CD3, CD4, CD5, CD8, CD10, CD19, CD22, HLA-DR, and TdT. Gene rearrangement analysis for monoclonality determination was carried out with restricted DNA (EcoR I and Hind III) hybridized with one of the following ^{32}P-labelled probes: T-cell receptor (TCR β), immunoglobulin heavy chain (JH), k light chain, and λ light chain. Phenotyping of the cell population from the excised lymph node (LN) revealed the presence of 66% B-cells and 35% T-cells. Most of the B cells (94%) expressed μ heavy chain only. Expression of both light chains was negligible (k = 7% and λ = 2%). Gene rearrangement, which indicates monoclonality, was positive on the level of TCR, Ig heavy chain, and both light chains. The data obtained suggests a neoplastic transforming event in lymphoid stem cells, which preceded the subsequent differentiation process into either B or T lymphoma. Am. J. Hematol. 56:219–223, 1997.

Key words: gene rearrangement; immunoglobulin gene; TCR gene; lymphoma; monoclonality; polyclonality

INTRODUCTION

Monoclonality of lymphocytes, either B or T, is a characteristic feature of lymphoproliferative diseases such as lymphoma, chronic lymphocytic leukemia, acute lymphoblastic leukemia, etc. This is usually due to malignant transformation of a rearranged progenitor cell, which occurs either on the level of the immunoglobulin or T-cell receptor genes. There are, however, rare but well-documented cases of biclonal lymphoproliferative diseases in which two clones of neoplastic cells proliferate concurrently or sequentially [1–6]. In the present report, we describe a case of a multi-monoclonal lymphoma with cells undergoing gene rearrangements on the level of TCR, Ig heavy chain, and both light chains.

CASE REPORT

A 73-year-old woman was admitted to our unit for evaluation of a right axillary mass. The mass was initially felt by the patient about 6 months prior to evaluation. The patient was asymptomatic, she had no weight loss, no fever, no night sweats, and her physical examination was normal except for a 7-cm right axillary mass. Laboratory tests including E.S.R., complete blood count including differential count, chemistry profile, and bone marrow aspiration and biopsy were normal. Thoracic and ab-

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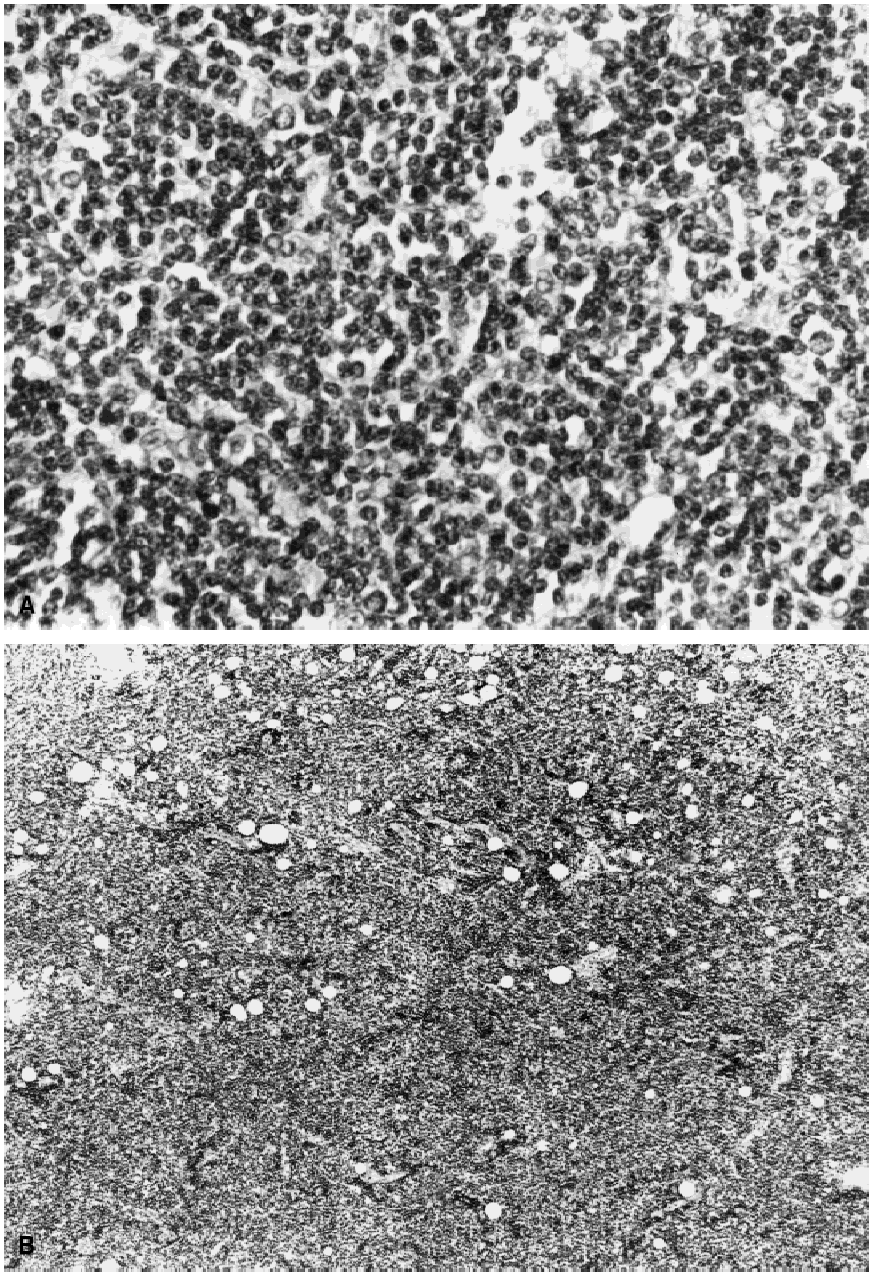

Fig. 1. Lymph node histological examination. Fat tissue effaced by an infiltrate composed predominantly of small, cleaved lymphoid cells with scattered large, non-cleaved lymphoid cells (H&E, ×200 and ×40).

dominal computed tomography scans showed no abnormalities except for the axillary lymphadenopathy.

A lymph node biopsy was diagnosed as malignant lymphoma, diffuse mixed small and large cell (Figs. 1 and 2, Working Formulation). The results of cell phenotyping and gene rearrangement analysis are described separately in detail (Table I and Fig. 3a–d).

The patient was treated with combination chemotherapy. Cyclophosphamid 400 mg/m^2, Doxorubicin 40 mg/m^2, Vincristine 1 mg, and Dexacort 12 mg were given on day 1 every 3 weeks. She received four courses of chemotherapy followed by radiation to the right axilla. The total dose of radiation was 4,400 cGy.

She responded well to treatment. Her last thoracic and abdominal computed tomography showed no lymphadenopathy or any other pathological findings. To date, there is no evidence of disease 2½ after completing therapy.

MATERIALS AND METHODS

Cell suspensions, in RPMI 1640 culture medium, from lymph node biopsies were prepared by mincing the specimens and passing them through a wire mesh. Cell phenotype was examined with immunofluorescence techniques (Coulter, EPICS-Profile II Flow Cytometer, Coulter, Luton, UK), using antibodies against Slg (IgM, IgA, IgD, and IgG), the kappa and lambda light chains,

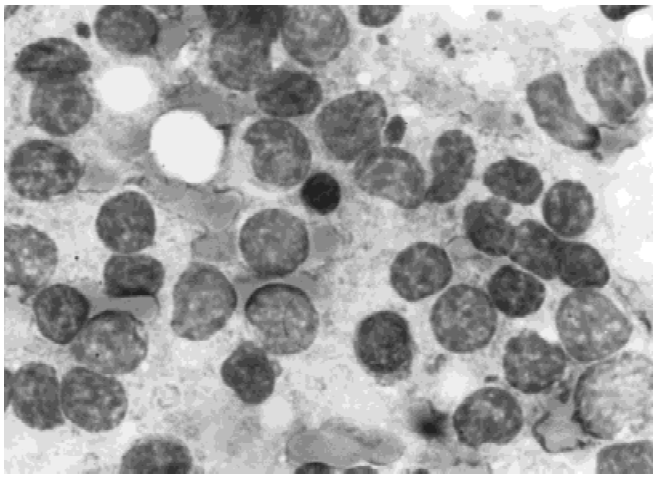

Fig. 2. Lymph node biopsy. Medium-sized neoplastic lymphatic cells with round-shaped or oval nuclei, finely reticulated chromatin, and narrow cytoplasm (Giemsa stain, ×100).

and monoclonal antibodies against CD3, CD4, CD5, CD8, CD10, CD19, CD22, CD25, HLA-DR, and TdT.

DNA extraction from LN cells was performed by the standard procedure. Ten micrograms of each DNA preparation was digested with EcoR I and Hind III restriction enzymes. Because of the unique pattern of bands obtained with Southern blotting, the whole gene rearrangement analysis process was repeated three times. First DNA digestion was started with 100 U of each enzyme, 2 hr later an additional amount of 100 U was added and the incubation continued for another 14 hr. The two other digestions were performed at the same time intervals with a double amount of enzymes. In all three digestions of the patient's DNA, human placenta DNA was used as control. DNA digestion was followed by electrophoresis on agarose gel and blotted onto Hybond-N^+ nylon membrane. The following nick-translated ^{32}P-labeled probes were used for Ig and TCR gene rearrangement analysis: (1) Ck 2.4 kb (EcoR I/SSp I); (2) Cl 3.6 kb (EcoR I/Hind III); (3) JH 5.6 kb (Oncor, Gaithersburg, MD); and (4) TCRβ 0.492 kb (Oncor). Hybridization with the labelled probes was carried out at 45°C for 18 hr using Hybrisol 1 (Oncor) as hybridization solution. The membrane was washed with 0.1 × SSC, 0.1% SDS solution (3 × 15 min each at room temperature plus 1 hr at 52°C) and autoradiographed at −70°C for 4–5 days with two intensifying screens. Before reuse with another probe, the membrane underwent stripping with 0.1 × SSC, 0.5% SDS solution (2 times for 15 min at 95°C) to remove the hybridized probe.

RESULTS

The phenotypic characteristics of cells obtained from the excised LN are revealed in Table I. They show a predominance of CD19, CD22 positive B-cells over CD3, CD5 positive T-cells (Table I). No double labelling of T- and B-cells was detected. The percentage of surface Ig correlated with the B-cell, CD19, CD22, markers. However 94% of them were μ chain positive and TdT was negative (Table I). Depending on the phenotypic characteristics, the percentage of surface λ and k light chain positive cells was too low to be indicative of monoclonality of B-cells.

The results of gene rearrangement analysis carried out with JH, TCRβ, Ck, and Cλ are described in Figure 3a–d. Gene rearrangements were shown in all the restriction enzyme lanes of the four probes.

DISCUSSION

Malignant hematologic disorders have been presumed to be of monoclonal origin. There are, however, rare but well-documented cases of biclonal lymphoproliferative diseases in which two clones of neoplastic cells proliferate concurrently or sequentially. Sklar et al. [2] and Duque et al. [6] reported rare cases of B-cell lymphoma with tumors composed of two subpopulations of cells, each expressing a different immunoglobulin molecule. Kornstein et al. [5] described a case with plasma cell neoplasm that later developed T-cell lymphoma, and Shimizu et al. [3] described a T-cell malignancy in a patient who was in remission from B-cell lymphoma. We have recently described a patient with non-Hodgkin T-cell lymphoma in whom B-cell monoclonal leukemia developed [7]. However, in acute lymphoblastic leukemia, Beishuizen et al. [8–10] described a multiband gene rearrangement on the level of IgH, Ig light chains, TCRβ, TCRγ, and TCRδ.

In the present case, the phenotypic characteristics of cells show that the LN cell population was comprised of two-thirds B-cells and one third T-cells. According to the membrane phenotype of T cells, it seems that they are polyclonal: all were CD3 positive and no double positive (CD4+/CD8+) cells were found (Table I). The high correlation between the B-cell markers (CD, 19–60%; CD, 22–67%) and the surface μ chain positivity (62%) reveals that the rearranged μ chain was expressed in all the B-cells. However, the expression of the light chain genes was negligible. Since the heavy chain is unlikely to be deposited in the surface membrane without the light chain, it was probably associated with light chain-like structure, the so-called surrogate light chains [11]. Although expression of light chain genes were very low, the use of Ck and Cλ probes revealed that the genes were rearranged. This, together with the presence of surface μ chain, CD22, CD19, and absence of CD10 and TdT, makes it possible that the B-cells were of the transitional pre-B type. Malignant transformation of transitional pre-

TABLE I. Cell Phenotypic Characteristics of the Lymph Node (LN) of the Patient

Markers (%)															
CD3	CD4	CD5	CD8	CD10	CD19	CD22	DR	Ig(t)	IgM	IgA	IgD	IgG	κ	λ	TdT
35	30	39	5	0	60	67	80	66	62	<1	<1	<1	7	2	0

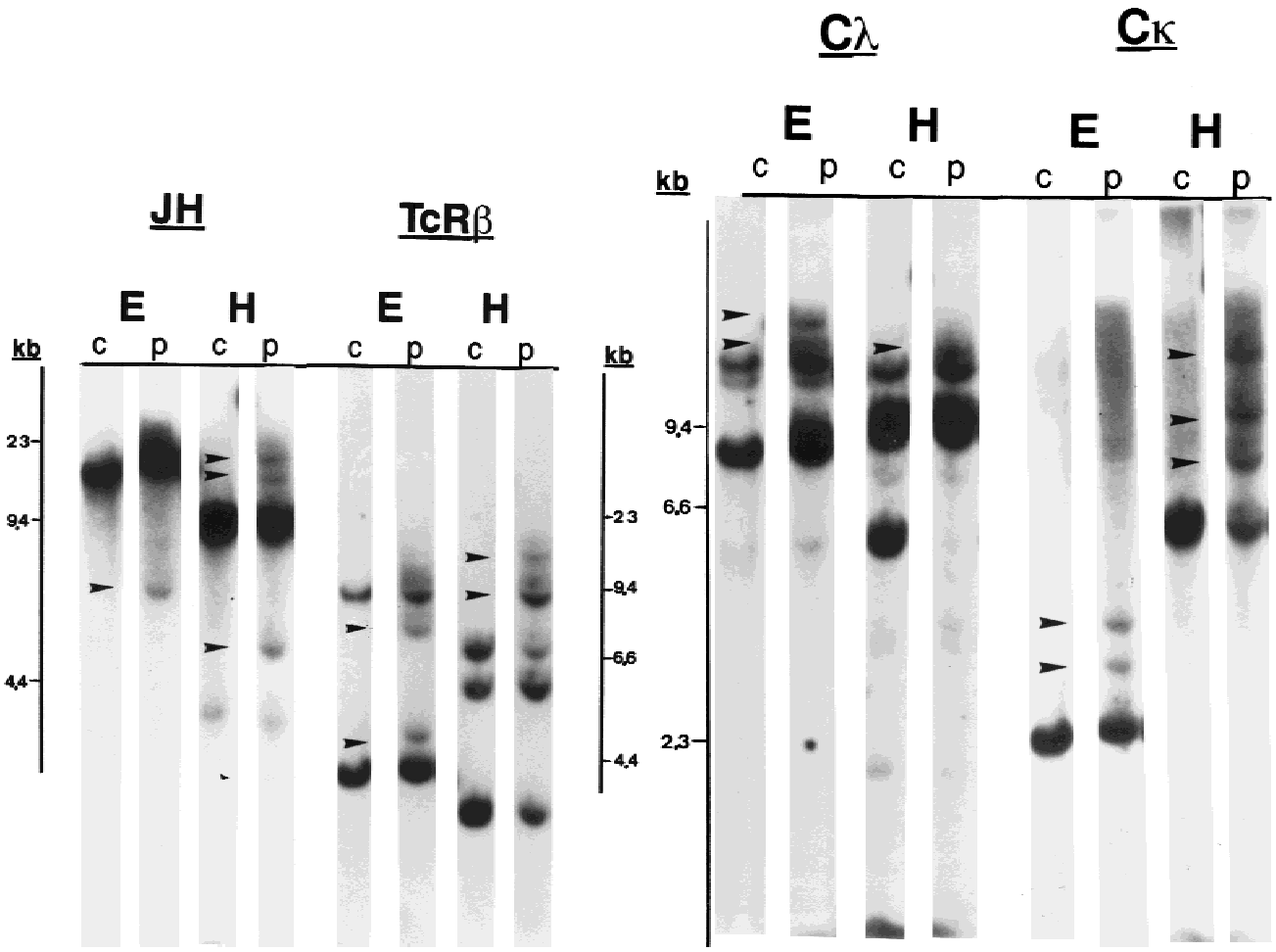

Fig. 3. Southern blotting of DNA extracted from lymph node cells. DNA samples of the patient's cells (p) and of human placenta (c) were digested with EcoR I (E) and Hind III (H) restriction enzymes. Hybridization was carried out with ^{32}P-labelled JH (a), TcRβ (b), Cλ (c), and Ck (d). Arrowheads mark non-germline bands.

B-cells has been recently recognised as defining a clinically unique type of ALL [12].

The gene rearrangement analysis with JH, Ck, and Cλ probes also revealed the presence of non-germline multibands. The fact that this pattern of gene rearrangement characterized each of the three repetitions of the analysis, ensures that the bands do not represent partial digestion products. The non-germline bands had different densities, indicating that the gene rearrangement was not biallelic but that the B-cells were divided into subclasses of monoclonal cells. The multi-band μ chain gene rearrangement pattern (Fig. 3a) warrants attention because it indicates the presence of at least three monoclonal μ chain rearranged subclasses. Since the heavy chain rearrangement is normally accompanied by rearrangement of one of the light chain genes, the division into B-cell subclasses becomes even more complex when the multiband gene rearrangements of the two light chain genes are taken into consideration.

Regarding TCR gene rearrangement, analysis with the TCR probe reveals a pattern with two non-germline band, when DNA was treated with either EcoR I or with Hind III (Fig. 3b). With respect to these results, it is possible that surface μ chain positive malignant B-cells may also have undergone TCR gene rearrangement (lineage infidelity) with no membrane expression of TCR

[13,14]. On the other hand, it is possible that in addition to the malignant B-cell population, monoclonal T-cell subclasses exist also.

Gene rearrangement of either the Ig or TCR genes occurs during ontogenesis at an early stage, a stage that usually precedes transformation, when such occurs. Transformed cells have the same gene rearrangement pattern as their progenitor cell, which is a marker for their monoclonality. Beishuizen et al. [8–10], when describing the multi-band gene rearrangement on the level of IgH, IgL, and the β, γ, and δ TCRs in acute lymphoblastic leukemia, showed that the pattern of gene rearrangement may change and that this change is associated with the duration of remission. This suggests that, with time, continuing Ig and TCR gene rearrangement processes may be taking place.

In summary, the unique multi-band gene rearrangement pattern on the level of Ig heavy chain, both Ig light chains, and TCR genes showed in this work, represented multistep and multilineage neoplastic developments. These developments were, probably, the result of continuing Ig and TCR gene rearrangement processes that occurred at some stage after transformation of an early lymphoid progenitor cell had taken place.

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